Human dendritic cell sequestration onto the *Necator americanus* larval sheath during ex-sheathing: a possible mechanism for immune privilege

A. Hassan[1], D.I. Pritchard[2], A.M. Ghaemmaghami[1]*

[1]Division of Immunology, School of Life Sciences, Faculty of Medicine & Health Sciences, [2]School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, United Kingdom

Running title: *Necator americanus* interaction with human dendritic cells

* Corresponding author: Life Sciences Building, Division of Immunology, School of Life Sciences, Faculty of Medicine & Health Sciences, University of Nottingham, Nottingham NG7 2RD, United Kingdom; Email: amir.ghaemmaghami@nottingham.ac.uk

ABSTRACT

Despite the profound health implications of *Necator americanus* (*N. americanus*) infection in humans, many aspects of its interaction with the host immune system are poorly understood. Here we investigated the early events at the interface of *N. americanus* larvae (L3) and human dendritic cells (DCs). Our data show that co-culturing DCs and the larvae triggers ex-sheathing of hookworms rapidly where a majority of DCs are sequestered onto the larval sheath allowing the ex-sheathed larvae to migrate away unchallenged. Intriguingly, DCs show negligible interaction with the ex-sheathed larvae, alluding to differences between the surface chemistry of the larva and its sheath. Furthermore, blocking of two key C-type lectin receptors on DC surface (i.e. DC-SIGN and mannose receptor) resulted in inhibition of ex-sheathing process and DC sequestration, highlighting the importance of C-type lectins on DCs in the induction of the ex-sheathing. Analyses of DC phenotype and cytokine profile after co-culture with the *N. americanus* larvae showed an immature phenotype as evidenced by the low expression of the maturation markers and cytokines. These data provide new insights into early events at the interface of human DCs and *N. americanus* larvae and could explain how L3 evade immune recognition upon initial interaction with DCs.

KEYWORDS: *Necator americanus*, dendritic cells, larval sheath, exsheathment, C-type lectin receptors, mannose receptor, immune modulation, immune evasion, hookworm

KEY FINDINGS

- Interaction between *Necator americanus* larvae and human DCs induces rapid ex-sheathing of larvae
- DCs are sequestered around the larval sheath whilst the ex-sheathed larval cuticle remains unchallenged
- The ex-sheathing process seems to be mediated by C-type lectins on the surface of DCs
- DCs sequestration around the sheath and unchallenged migration of larvae could explain the inefficiency of immune responses against *Necator americanus*

INTRODUCTION

Despite its profound health implications, chronicity and significant public health burden in developing countries, many aspects of human *N. americanus* infection, particularly early events at the interface with the host immune system, are under researched (Quinnell *et al.* 2004, Loukas and Prociv, 2001, Hotez *et al.* 2008). These insidious parasites infect and re-infect, following which no efficient immunological memory develops in the host, rendering chemotherapeutic treatment as the method of choice, which is also inefficient due to the high prevalence of re-infection.

Efforts in developing more effective therapeutic approaches could be helped by a better understanding of the initial interactions between *N. americanus* larvae and key components of the innate immune system such as dendritic cells (DCs). DCs are sentinels of the immune system and act as a bridge between the innate and adaptive immune systems (Savina and Amigorena, 2007). DCs are abundant in all barrier tissues (e.g. skin and airway epithelium) and equipped with a range of pattern recognition receptors (PRRs) on their surface (e.g. Toll-like and C-type lectin receptors (CLRs)) that can recognise various pathogen associated molecular

patterns (PAMPs)(Salazar *et al.* 2013). Interestingly previous studies have identified a range of lectins isolated from plants capable of binding to sugars present on the *N. americanus* L3 sheath, including mannose, fucose, heparan sulphate and galactose (Kumar and Pritchard, 1992a) which could potentially act as ligands for CLRs on DCs.

During its life cycle *N. americanus* has many opportunities to interact with the host DCs (Quinnell *et al.* 2004, Geiger *et al.* 2007). As part of their armoury of PRRs, DCs express a range of CLRs with specificity for the recognition of glycosylated proteins (Thompson *et al.* 2011, Salazar *et al.* 2013). Amongst CLRs expressed by DCs are dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) and mannose receptor (MR), both of which have been implicated in key DC functions including mediating immune responses to different pathogens as well as immune modulation (Garcia-Vallejo and van Kooyk, Geijtenbeek *et al.* 2002, Wollenberg *et al.* 2002, Salazar *et al.* 2013, Emara *et al.* 2011, Emara *et al.* 2012, Royer *et al.* 2010). However, the biological relevance of the glycosylated *N. americanus* sheath in the context of interaction with CLRs on DCs has not been investigated.

The antigen presenting cell function of dendritic cells directly depends on their ability to migrate to the site of infection (Martin-Fontecha *et al.* 2009). Upon capturing pathogens, DCs migrate to lymph nodes where processed antigens are presented to naïve T cells, in the context of MHC molecules, leading to polarisation of T cells towards distinct functional subsets such as Th1, Th2, Th17 and regulatory T cells (Smith-Garvin *et al.* 2009). Many microorganisms have developed strategies (e.g. masking of PAMPs or inducing changes in PRR expression) to evade efficient recognition by DCs (van Kooyk and Geijtenbeek, 2003). In addition, any physical or chemical barrier that interfere with DC migrating to or from the site of infection could also hamper mounting appropriate immune responses. This function is indispensable for the maintenance of immune surveillance and tissue homeostasis as well as initiating protective tolerogenic and pro-inflammatory responses (Imai *et al.* 2012).

In this study we have investigated the cross-talk between human DCs and *N. americanus* larvae, in particular the biological significance of the glycosylated molecules on the *N. americanus* L3 sheath in influencing DCs function and their interaction with the *N. americanus* larvae.

MATERIALS AND METHODS

All materials were purchased from Sigma-Aldrich, U.K., unless otherwise stated

Preparation and identification of *N. americanus* (L3) larvae

Infective *N. americanus* larvae were cultured from faecal material derived from infected individuals as described previously (Kumar *et al.* 1992). The larvae were deemed to be axenic following microbiological analysis (FDAS, BioCity, Nottingham).

Dendritic cell generation

Monocyte derived dendritic cells (DCs) were generated from peripheral blood monocytes which were obtained from human blood buffy coats after obtaining written informed consent and approval of local Ethics Committee (National Blood Transfusion Service, U.K., 2009/D055) as we have previously described (Chau *et al.* 2013). Briefly, the peripheral blood mononuclear cells (PBMCs) were isolated via histopaque density gradient centrifugation. Monocytes were then isolated out from the PBMC by incubating the suspension with CD14+ magnetic beads (Milteny Biotech, U.K.) obtaining a purity of >98% as we have described before (Garcia-Nieto *et al.* 2010). Subsequently, purified CD14+ monocytes were cultured with complete RPMI medium (10% Fetal Bovine Serum, 2Mm L-glutamine, 1% Penicillin/Streptomycin and 1% non-essential amino acid solution) supplemented with 50 ng/mL GM-CSF and 250 IU/ml IL-4 in a 24 well plate for a period of 6 days to generate

112 immature DCs (Salazar *et al.* 2016).

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114 **_N. americanus_ (L3) incubation with DCs**

115 Immature DCs were cultured in complete RPMI media (10% Fetal Bovine Serum, 2Mm L-
116 glutamine, 1% Penicillin/Streptomycin and 1% non-essential amino acid solution) and
117 incubated with approximately 50 ensheathed *N. americanus* L3 larvae for 24-hours. During the
118 incubation period the samples were imaged using the ZOE™ Fluorescent Cell Imager (Biorad).

119

120 **CLRs blocking experiments**

121 To assess the potential involvement of specific CLRs in DC- hookworm interaction, immature
122 DCs were treated with either 20 µg/ml of blocking antibodies for DC-SIGN (clone H-200) and
123 MR (clone 15.2) or mannan (from *Saccharomyces cerevisiae*) (100µg/ml) for 25 minutes at
124 370C prior to addition of approximately 50 *N. americanus* larvae. The cells were then incubated
125 for a further 24-hours at 370C/5% CO2 and the samples were imaged using the ZOE™
126 Fluorescent Cell Imager. This was compared to DCs treated with the appropriate isotype
127 controls (rabbit IgG and normal mouse IgG1) and DCs which were untreated; both conditions
128 were also incubated with the infective larvae. All antibodies purchased from Santa Cruz
129 Biotechnology.

130

131 **_N. americanus_ (L3) incubation with conditioned DC media**

132 To assess the effect of DCs secretions on larvae ex-sheathing, DCs were stimulated with either
133 mannan (100µg/ml) or Lipopolysaccharide (LPS) (100ng/ml) from *Escherichia coli* (*E. coli*)
134 for 24-hours at 370C/5% CO2. The cell free conditioned supernatant was added to
135 approximately 50 *N. americanus* larvae for 24-hours. Following the incubation period, the
136 samples were imaged using an inverted Microscope (Olympus CKX41, Olympus America) and

analysed with Lumenera Infinity Capture software. This was compared to the supernatant from untreated DCs incubated with the infective larvae.

Staining for cell surface markers and quantifying DC viability

In order to prepare the DCs for phenotype analysis via flow cytometer, the cells were harvested and washed twice in PBA buffer (5% Albumin solution from bovine serum, 0.1% Sodium azide in PBS). The desired antibodies (e.g. CD11c, CD14 and CD83) were added to the pellet, vortexed and incubated for 20 minutes, in dark at 4 0C. Nonreactive isotype-matched antibodies and unstained cells were used to determine non-specific staining. The samples were then washed with PBA and finally fixed with paraformaldehyde solution (0.5% in PBS). This was stored at 4^{o} C to be analysed within a 7 day period.

The viability of DCs were analysed using the ANNEXIN V– FITC Kit-Apoptosis Detection Kit (Beckman Coulter) according to the manufacturer's protocol. Expression of surface markers and the level of Annexin-V and Propidium Iodide expression in DCs were assessed via flow cytometery analysis (Cytomics FC 500, Beckman Coulter) with a minimum of 20,000 events collected for each sample. The data obtained were analysed using the Weasel V.2.7.4 software. Median fluorescence intensity and percentage of positive cells for each marker was determined and further evaluated using GraphPad Prism 6 analysis software.

Cytokine expression

The levels of cytokines were measured with ELISA Kits and were analysed according to the manufacturer's protocol. All samples were analysed in two duplicates. Absorbance was measured at 450 nm with SpectraMax Paradigm. IL-1β (200-01B), TNFα (900-K25), IL-10 (900-K21), IL-12 (900-K96) and IL-6 (900-K16) were purchased from PeproTech and IL-8 (DY208) from R&D Systems.

Statistical analysis

The means and ±SEM are shown. The statistical significance of the data was analysed and evaluated using Student's t test with GraphPad Prism 6 analysis software. Statistical significance was determined using the Holm-Sidak method with a p value of ≤ 0.05.

RESULTS

Ex-sheathing of *N. americanus* (L3) larvae upon co-culture with immature dendritic cells

To assess the consequence of a physical interaction between DCs and *N. americanus*, immature DCs were incubated with 50 *N. americanus* L3 larvae. The interactions were imaged and monitored using the ZOE™ Fluorescent Cell Imager (BioRad) for up to 24-hours. Microscopy data showed that upon contact with the larval sheath, DCs are sequestered on the surface of larval sheath which in turn triggered ex-sheathing, whereby the larvae discarded their outer cuticle. Ex-sheathing in this study is defined by the initial breach and emergence of the larva from its sheath. This phenomenon is observed at variable intervals after a minimum of 1 hour incubation with DCs and complete ex-sheathing (i.e. full length larva leaving the cuticle) could take up to 4 hours. Notably, DCs in direct contact with the sheath seem to attract a majority of bystander cells leading to formation of large DC aggregates and sequestration of DCs around the sheath, alluding to an adhesion cascade. Upon full ex-sheathing the larvae migrate away from the sheath with the sequestered DCs, as well as free DCs, exhibiting negligible interaction with the ex-sheathed larvae. The ex-sheathing of a single hookworm was examined, documenting this novel interaction (Figure 1) (Supplementary Video 1).

Following 24 hours, DCs form dense aggregates around the sheath and remain un-attracted to the exposed larvae. Despite highly dense cell aggregates surrounding discarded sheaths they can still be visualised within some aggregates. (Figure 2).

Blocking CLRs on DC surface and *N. americanus* (L3) treatment with conditioned media

Previous research has distinguished *N. americanus* from other hookworm species based on its distinct glycosylated surface chemistry, which binds a range of lectins derived from plants (Kumar and Pritchard, 1992a). It was therefore reasonable to assume that sugars on the worm sheath could meditate the interaction with DCs. To investigate this possibility, we blocked DC-SIGN and MR, 2 major C-type lectins expressed by human DCs, using specific blocking antibodies or mannan (to partially saturate MR and DC-SIGN) before incubation with *N. americanus* larvae for 24 hours as described earlier. Our data clearly show almost complete abrogation of DC aggregation around *N. americanus* larvae in the presence of either α-DC-SIGN, α-MR or mannan compared to untreated DCs (Figure 3a). In parallel DCs treated with the respective isotype control antibodies were also examined which illustrated no changes in DC aggregation compared to untreated DCs (data not shown).

To elucidate whether soluble factors produced by DCs play a role in the observed ex-sheathing, DCs were stimulated with mannan (100μg/ml) (to simulate CLR ligation) and the conditioned media was collected after 24 hours. The *N. americanus* larvae were then incubated with the conditioned media for 24 hours as described. In parallel, media from unstimulated DCs was collected and incubated with the larvae as a control. The hookworms did not ex-sheath in culture with neither the conditioned media nor media collected from un-stimulated DCs, suggesting that a cell mediated interaction is required for the induction of ex-sheathing (Figure 3b).

DC surface phenotype in response to *N. americanus* (L3)

To better understand the effect of *N. americanus* larvae on DCs function we assessed DCs phenotype after 24 hours incubation with *N. americanus* larvae. In control cultures DCs were

stimulated with 100 ng/mL LPS to induce maturation. In this study we report that DCs retrieved from co-culture with viable axenic larvae maintained an immature phenotype as evidenced by a lack of up-regulation in maturation markers CD80, CD83, CD86, CD40 and HLA-DR. In addition, there was a significant downregulation in CD206 expression (Figure 4). Subsequently, the ability of DCs to acquire a mature phenotype in response to co stimulation with LPS in the presence of *N. americanus* larvae was assessed. These data showed DCs treated with *N. americanus* larvae will remain responsive to LPS stimulation (Figure 5). In all these experiments we monitored DC viability using Annexin-V and PI staining and did not observe any significant changes in DCs viability upon co-culture with *N. americanus* larvae (Figure 6).

DC cytokine expression in response to *N. americanus* (L3) and LPS stimulation

Following the stimulation of DCs with either the infective L3 *N. americanus* larvae, LPS or both, supernatant samples were collected at 24 hours and were analysed for IL6, 8, 10 and 12 using ELISA. Our data show that while axenic *N. americanus* larvae on its own do not induce any cytokine production by DCs, they seem to suppress LPS induced cytokine production however these changes were not statistically significant (p value ≤ 0.3) (Figure 7).

DISCUSSION

Infection with *N. americanus* has remained a major health problem with significant health implications. The high prevalence of reinfection, due to inefficient protective immunity, makes disease eradication a challenge. Therefore better understanding of how the immune system interacts with infective larvae could pave the ways for the rational design of novel treatment strategies. This study provides new insights into early immunological events at the interface of human DCs and *N. americanus* larvae and could explain the lack of efficient immune response during early stages of infection.

The ex-sheathing of *N. americanus* larvae has been observed during the initial stages of infection as well as in the presence of human sweat (Pasuralertsakul and Ngrenngarmlert, 2006, Hawdon *et al.* 1993, Matthews, 1982), however the factors initiating ex-sheathing and the benefit of this to the parasite are yet to be fully understood (Loukas and Prociv, 2001). From previous literature, it is known that the larval sheath does not accompany the hookworm post the skin stages of infection and progression into the blood circulation (Kumar and Pritchard, 1992b); implying the ensheathed larvae encounter immune cells in the skin *in vivo*.

Our data show for the first time that immature DCs bind to the ensheathed larvae, initiating the *N. americanus* to ex-sheath and mechanically migrate away from this site leaving behind its sheath. DCs are sequestered onto the discarded sheath and continue to form aggregates, exhibiting no interest in binding to the exposed larval cuticle allowing its unchallenged movement away from DCs. Migration of immature DCs from the site of infection to draining lymph nodes, where they interact with naïve T cells, is a key step in initiating an efficient immune response (Heuze *et al.* 2013). Therefore, it is reasonable to suggest that DC sequestration on larval sheath and no interaction between DCs and ex-sheathed larvae could play a major role in the lack of immune recognition/activation at early stages of infection.

The differential interaction between DCs and the larval sheath versus the larvae allude to a disparity between the surface chemistry of the sheath and the larvae. While the exact nature of differences in the surface properties of the larvae and sheath are yet to be fully characterised, these data clearly indicates the presence of distinct chemical signatures on the *N. americanus* sheath that attract DCs. Interestingly earlier work by authors has identified a range of sugars on the L3 sheath, including mannose, fucose, heparan sulphate and galactose (Kumar and Pritchard, 1992a). These sugars could be clear targets for a range of C-type lectin receptors expressed on the surface of immature DCs that are part of DCs armoury of Pattern Recognition Receptors (PRRs) (Thompson *et al.* 2011).

Amongst the CLRs expressed by DCs are DC-SIGN and mannose receptor (MR or CD206) both of which have been implicated in mediating immune responses to different pathogens as well as immune modulation (Garcia-Vallejo and van Kooyk, Geijtenbeek *et al.* 2002, Wollenberg *et al.* 2002, Salazar *et al.* 2013). Given the high expression of DC-SIGN and MR on myeloid DCs and their prominent role in recognition of different pathogens, we investigated their potential role in DCs interaction with *N. americanus* live larvae by blocking MR and DC-SIGN. Our data illustrate that DCs treated with blocking antibodies against DC-SIGN and MR or mannan (a ligand for both DC-SIGN and MR) (Salazar *et al.* 2013) exhibit a significant decrease in induction of larvae ex-sheathing and forming DC aggregates around larval sheath, compared to untreated DC. These data clearly indicate a role for MR and DC-SIGN in mediating interactions between DC and the larval sheath. Additionally, the treatment of *N. americanus* larvae with conditioned media from DCs stimulated mannan, an agonist for both MR and DC-SIGN, showed that the hookworms do not ex-sheath in response to cell free conditioned media, proposing a cell mediated interaction and that binding to CLRs on DC are necessary for induction of hookworms ex-sheathing.

Previous studies have shown the ability of some pathogens to subvert DC-SIGN function in order to evade immune detection and surveillance; these include viral pathogens, such as HIV-1 via gp120 protein, and non-viral pathogens including Mycobacterium tuberculosis (van Kooyk and Geijtenbeek, 2003, Ludwig *et al.* 2004). However, in the context of this study, the *N. americanus* uses interaction with both DC-SIGN and MR to sequester DCs onto its sheath. This could provide a plausible explanation for the poor T cell responses observed *in vivo*, as the sequestration of DCs on larval sheaths could prevent necessary DC migration to lymph nodes that is a perquisite for developing an effective adaptive immune response (Martin-Fontecha *et al.* 2009). These data provide strong rationale for further experiments (e.g. targeting specific lectins on larvae surface and/or silencing MR and DC-SIGN

expression on DCs)(Royer *et al.* 2010, Emara *et al.* 2012) in order to fully elucidate the cross-talk between the sugar moieties on larvae sheath and the CLRs on DCs.

Another interesting observation arising from these experiments was a significant reduction in MR expression upon co-culture with *N. americanus* larvae while all the other tested surface markers did not change and DCs maintained an immature phenotype. The immature phenotype of DCs is perhaps reflection of the axenic nature of larvae that are used in these experiments which is unlikely to be the case *in vivo*. Nevertheless, the down-regulation of MR expression on DCs in co-culture with *N. americanus* is of interest and further highlights the presence of MR ligands on larval sheath. This observation is in line with other studies in *Schistosoma mansoni* infection, revealing a novel pathway involving the internalization (and decrease in MR expression) of helminth derived glycoproteins through the MR. This interaction has been shown to interfere with DC protein synthesis, conditioning DCs to support a Th2 phenotype differentiation (Everts *et al.* 2012).

To further investigate whether the larvae are able to modulate DC responses to other stimuli we also studied DC cytokine profile after co-stimulation with LPS from E.coli, which is likely to be present during any *in vivo* exposure. Interestingly, DCs co-cultured with *N. americanus* larvae maintained their ability to respond to LPS stimulation as evidenced by upregulation in maturation markers. Furthermore, our data clearly show a general suppression in LPS induced cytokine (IL-6, IL-8, IL-10 & IL-12) production in the presence of larvae which was not due to any changes in DC viability. Although such suppression in cytokine production was not statistically significant (p value ≤ 0.3), most likely due to small number of donors, it is in line with the generation of non-immunogenic or poorly immunogenic DCs.

In summary, our data clearly suggest that the *N. americanus* larvae actively target DC-SIGN and MR on DCs that leads to DC sequestration on the surface of larval sheath and unchallenged migration of un-sheathed larvae enabling larvae to escape immune surveillance

and potentially promote pathogen survival (figure 8). These data provide new insights into the early events at the interface of DCs and *N. americanus* larvae which could pave the way for the rational design of new and more efficient intervention strategies against hookworm infection.

ACKNOWLEDGEMENTS

Authors would like to thank Dr Gary Telford (School of Pharmacy, University of Nottingham) for preparing the infective L3 *Necator americanus* larvae.

FINANCIAL SUPPORT

The production of the axenic larvae used in this work was supported by an MS Society grant.

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Figure 1: The mechanical interaction of immature DCs with *Necator americanus*. The sheathed larva remains dormant during the initial incubation period where DCs are sequestered onto its sheath (up to approx.1 hour). Subsequently the en-sheathed larva begins to move dynamically until it finally breaches its sheath (A). Once the larvae begin ex-sheathing, the process occurs within seconds as the sheathed hookworm exits the cuticle sheath swiftly. For this individual larva ex-sheathing was initiated at the 4-hour time point. At the first sight of ex-sheathing, images were captured sequentially with 10 second intervals (B: 10 seconds; C: 20 seconds). The ex-sheathed hookworm progressively migrates away from its discarded cuticle sheath with sequestered DCs (H). Images are representative of 3 independent experiments using DCs from 3 different donors and 3 larvae batches. The sheathed larva remains dormant during the initial incubation period where DCs are sequestered onto its sheath (up to approx.1 hour). Subsequently the en-sheathed larva begins to move dynamically until it finally breaches its sheath (A). Once the larvae begin ex-sheathing, the process occurs within seconds as the sheathed hookworm exits the cuticle sheath swiftly. For this individual larva ex-sheathing was initiated at the 4-hour time point. At the first sight of ex-sheathing, images were captured sequentially with 10 second intervals (B: 10 seconds; C: 20 seconds). The ex-sheathed hookworm progressively migrates away from its discarded cuticle sheath with sequestered DCs (H). Images are representative of 3 independent experiments using DCs from 3 different donors and 3 larvae batches.

Figure 2. Dendritic cell sequestration around *N. americanus* larvae sheath. Following 24-hours majority of DCs form dense aggregates around discarded larval sheath. Data show representative images of 6 independent experiments.

Figure 3. The impact of blocking CLRs on DCs interaction with *N. americanus.* (A) Microscopy data illustrates that DCs treated with either α-DC-SIGN, α-MR or mannan prior to incubation with *N. americanus* exhibit a significant decrease in aggregation following 24-hour

incubation with the larvae compared to untreated DCs where DCs form aggregates around larvae (also shown in figure 2). (B) *N. americanus* larvae do not ex-sheath in culture with 'cell-free' conditioned media from DCs stimulated with mannan. Data show representative images of 3 independent experiments.

Figure 4. Dendritic cells maintain an immature phenotype upon interaction with N. *americanus*. Dendritic cells were cultured in the presence of *N. americanus* larvae for 24 hours followed by assessing the expression of co-stimulatory receptors/maturation markers CD40, CD80, CD83, CD86, CD206 (mannose receptor), CD209 (DC-SIGN) and HLA-DR using flow cytometry. Data show no changes in the expression of CD40, CD80, CD83, CD86 and HLA-DR compared to un-stimulated cells which is in line with an immature phenotype. While there are no changes in CD209 expression levels in response to *N. americanus* larvae, there is a significant down regulation in CD206 expression. Cells stimulated with LPS show an increase in the expression of maturation markers as expected. Data shown are mean values ± SD of 3 independent experiments using blood samples from 3 different donors.

Figure 5. *N. americanus* larvae does not modulate the dendritic cells response to LPS stimulation. Dendritic cells were simultaneously stimulated with LPS and *N. americanus* larvae for 24 hours followed by assessing the expression of co-stimulatory receptors/maturation markers CD40, CD80, CD83, CD86, CD206 (mannose receptor), CD209 (DC-SIGN) and HLA-DR using flow cytometry. Data indicate that dendritic cells remain responsive to LPS stimulation when co-stimulated with *N. americanus* larvae. Data shown are mean values ± SD of 3 independent experiments using blood samples from 3 different donors.

Figure 6: The viability of dendritic cells post treatment with *N. americanus* larvae. Dendritic cells viability shows no significant changes after 24 hour treatment with the *N. americanus* larvae with >80% viability quantified by measuring the expression levels of Annexi-V and Propidium Iodide (PI). Cells in lower left quadrant are negative for both

Annexin-V and PI which indicates viability. Data shown is representative of 3 independent experiments using blood samples from 3 different donors.

Figure 7. The cytokine profile of dendritic cells in response to *N. americanus* in the presence and absence of LPS stimulation. *N. americanus* infective larvae on their own did not induce production of selected pro (IL-6, IL-8, IL-12) or anti-inflammatory (IL-10) cytokines. However, cytokine production in response to LPS seems to be ameliorated when dendritic cells are stimulated with LPS and *N. americanus simultaneously.* Such suppression was consistently observed in all cytokines tested but did not reach statistical significance expression (p value= ≤0.3 Data shown are mean values ± SD of 3 independent experiments using blood samples from 3 different donors.

Figure 8: The proposed mechanism of *Necator americanus* immune evasion strategy. Dendritic cells bind the *N. americanus* sheath via CLRs which triggers the ex-sheathing of the larvae. Dendritic cells are then sequestered onto the discarded sheath and the larvae escapes to the vasculature unchallenged.

Supplementary Video 1. The mechanical interaction of immature DCs with *N. americanus.* This short film describes the novel interactions between DC and *N. americanus*. Representative of 3 independent experiments.